

CLAIMS

What is claimed is:

1. A method of screening a natural sample for an affinity ligand that binds to a protein target, comprising:

(1) mixing a protein target and a natural sample in solution to form a reaction mixture;

(2) incubating the reaction mixture under conditions allowing complex formation by the target and any target-binding ligand present in the sample;

(3) passing the reaction mixture through a first size-exclusion medium that removes from the reaction mixture any small molecular weight compounds each having a molecular weight less than a first preset value;

(4) subjecting the size-excluded reaction mixture from step (3) to conditions promoting dissociation of any ligand/target complex into free ligand and free target; and

(5) passing the reaction mixture resulting from step (4) through a second size exclusion medium that removes from the reaction mixture any molecule larger than a second preset value.

2. The method of claim 1, wherein the first size-exclusion medium removes molecules having a molecular weight of about 2,000 daltons or less.

3. The method of claim 1, wherein the first size-exclusion medium removes molecules having a molecular weight of about 1,500 or less.

4. The method of claim 1, wherein the first size-exclusion medium comprises a gel filtration or size exclusion HPLC column.

5 5. The method of claim 1, wherein step (4) comprises adding to the size-excluded mixture from step (3), a solution comprising an organic solvent and an organic acid.

10 6. The method according to claims 1, 4, or 5, wherein the second size-exclusion medium comprises an ultrafiltration membrane.

15 7. The method according to claims 1, 4, or 5, wherein the second size-exclusion medium removes from the reaction mixture, molecules having a molecular weight of about 10,000 daltons or more.

20 8. The method according to claims 1, 4, or 5, wherein the second size-exclusion medium removes from the reaction mixture, molecules having a molecular weight of about 3,000 daltons or more.

25 9. The method according to claims 1, 4, or 5, wherein the second size-exclusion medium removes from the reaction mixture, molecules having a molecular weight of about 2,000 daltons or more.

30 10. The method of claim 6, wherein the ultrafiltration membrane removes from the reaction mixture, molecules having a molecular weight of about 10,000 daltons or more.

11. The method of claim 6, wherein the ultrafiltration membrane removes from the reaction mixture, molecules having a molecular weight of about 3,000 daltons or more.

5 12. The method of claim 6, wherein the ultrafiltration membrane removes from the reaction mixture, molecules having a molecular weight of about 2,000 daltons or more.

10 13. The method according to claims 1, 4, or 5, further comprising:

(7) comparing the analytical results of step (6) with a reference standard.

15 14. The method of claim 13, wherein the reference standard comprises the analytical results of subjecting either a sample of the protein target alone or a mixture of the protein target with a non-target-binding natural sample, to steps (2)-(6).

20 15. The method according to claims 1, 4, or 5, further comprising, in step (1), including a known competitive ligand that binds to the target in the reaction mixture prior to step (2).

25 16. The method of claim 15, wherein the concentrations of the known competitive ligand and the target are approximately equimolar.

30 17. The method of claim 15, wherein the known competitive ligand concentration is within a range of approximately twice to 10 times the target concentration.

5

(7) comparing the analytical results of step (6) with a reference standard.

15

20

(6) ~~subjecting the reaction mixture resulting from step (5), to at least one structural or functional analysis.~~

25

30

WSGH Docket No. HKI-106AX
WEINGARTEN, SCHURGIN,
GAGNEBIN & HAYES, LLP
TEL. (617) 542-2290